IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: David WALLACH et al

Application No.: 08/485,129

Filed: June 7, 1995

For: ISOLATED DNA ENCODING TUMOR NECROSIS FACTOR BINDING PROTEIN II, ...

Art Unit: 1644

Examiner: R. Schwadron

Washington, D.C.

Atty.'s Docket: WALLACH=5B

Date: September 25, 2001

THE COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

Transmitted herewith is a [ ] Amendment [X] Brief on Appeal (triplicate); Appendices A-C in the above-identified application.

[ ] Small Entity Status: Applicant(s) claim small entity status. See 37 C.F.R. §1.27.

[XX] The fee has been calculated as shown below:

	(Col. 1) CLAIMS REMAINING AFTER AMENDMENT		(Col. 2) HIGHEST NO. PREVIOUSLY PAID FOR	(Col. 3) PRESENT EXTRA EQUALS	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
TOTAL	*	MINUS	** 20	0	x 9	$		x 18	$
INDEP.	*	MINUS	*** 3	0	x 40	$		x 80	$
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM					+ 135	$		+ 270	$
					ADDITIONAL FEE TOTAL	$	OR	TOTAL	$

* If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.

** If the "Highest Number Previously Paid for" IN THIS SPACE is less than 20, write "20" in this space.

*** If the "Highest Number Previously Paid for" IN THIS SPACE is less than 3, write "3" in this space.

The "Highest Number Previously Paid For" (total or independent) is the highest number found from the equivalent box in Col. 1 of a prior amendment of the number of claims originally filed.

[XX] Conditional Petition for Extension of Time

If any extension of time for a response is required, applicant requests that this be considered a petition therefor.

[XX] It is hereby petitioned for an extension of time in accordance with 37 CFR 1.136(a). The appropriate fee required by 37 CFR 1.17 is calculated as shown below:

Small Entity			Other Than Small Entity		
Response Filed Within			Response Filed Within		
[ ]	First	- $ 55.00	[ ]	First	- $ 110.00
[ ]	Second	- $ 195.00	[ ]	Second	- $ 390.00
[ ]	Third	- $ 445.00	[XX]	Third	- $ 890.00
[ ]	Fourth	- $ 695.00	[ ]	Fourth	- $ 1390.00
Month After Time Period Set			Month After Time Period Set		

[ ] Less fees ($_____) already paid for ___ month(s) extension of time on _____.

[XX] Fee for Filing Brief on Appeal: $310.00

[ ] Please charge my Deposit Account No. 02-4035 in the amount of $_____.

[XX] Credit Card Payment Form, PTO-2038, is attached, authorizing payment in the amount of $ 1,200.00.

[ ] A check in the amount of $_____ is attached (check no.).

[XX] The Commissioner is hereby authorized and requested to charge any additional fees which may be required in connection with this application or credit any overpayment to Deposit Account No. 02-4035. This authorization and request is not limited to payment of all fees associated with this communication, including any Extension of Time fee, not covered by check or specific authorization, but is also intended to include all fees for the presentation of extra claims under 37 CFR §1.16 and all patent processing fees under 37 CFR §1.17 throughout the prosecution of the case. This blanket authorization does not include patent issue fees under 37 CFR §1.18.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

ATTY.'S DOCKET: WALLACH=5B

In re Application of:

David WALLACH et al

Appln. No.: 08/485,129

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For: ISOLATED DNA ENCODING TUMOR NECROSIS FACTOR BINDING PROTEIN II, AND VECTORS, HOSTS AND PROCESSES USING SUCH DNA

Art Unit: 1644

Examiner: R. Schwadron

Washington, D.C.

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BRIEF ON APPEAL

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith is applicant's Brief on Appeal in triplicate.

The present appeal is taken from the action of the examiner in finally rejecting claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64. The full text of claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64 under appeal appears in Appendix A attached hereto.

REAL PARTY IN INTEREST

The present application is owned by Yeda Research and Development Co. Ltd., which is the research and development arm

of the Weizmann Institute of Science in Rehovot, Israel. The exclusive licensee of the present invention is Inter-Lab Limited, an Israeli company of Ness-Ziona, Israel. Inter-Lab Limited is a subsidiary of InterPharm Laboratories Limited, an Israeli company of Ness-Ziona, Israel, which is a subsidiary of Ares Serono N.V., whose parent company, Ares Serono S.A., is a holding company under which there are many subsidiaries worldwide.

RELATED APPEALS AND INTERFERENCES

Appellant is aware of no other appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the present appeal.

The present application is a divisional of parent application 07/930,443, filed August 19, 1992. All of the claims in the present case are drawn to invention(s) deleted from the parent application in light of a restriction requirement. The claims remaining in said parent application drawn to the TBP-II protein are now involved in an interference proceeding with the claims of U.S. patent 5,344,915. This is pending interference no. 103,625 under the name Wallach v LeMaire. A final decision has been rendered by the Board of Patent Appeals and Interferences, which decision is currently on appeal to the United States District Court for the District

of Columbia under the name Goliath Hundertzehnte Vermoegensverwaltungsgesellschaft-mbH v Yeda Research and Development Co. Ltd., Case No. 00-1720 EGS. While it is not believed that this interference will directly affect or be directly affected by or have a bearing on the Board's decision in the present appeal, it is nevertheless being brought to the Board's attention as it is related in the sense discussed above, and the examiner has required that reference to this interference be made in the present section of the appeal.

STATUS OF CLAIMS

Claims 11-14, 35-39 and 43-64 presently appear in this case. Claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64 are under final rejection. Claims 1-10, 15-34 and 40-42 have been cancelled. Claims 14, 39, 45, 50, 55 and 62 have been withdrawn from consideration, but it is understood that in the event that the claims on appeal are found allowable, these withdrawn claims will be treated as per MPEP §821.04.

STATUS OF AMENDMENTS

A final rejection was issued in this case on January 30, 2001. Aside from the Notice of Appeal, no papers have been filed subsequent thereto.

SUMMARY OF THE INVENTION

The present invention is directed to isolated DNA molecules which encode Tumor Necrosis Factor (TNF) Binding Protein II (TBP-II) (page 1, lines 2-6). The protein encoded by the DNA of the present invention was initially isolated from human urine and was found to have the ability of selectively inhibiting the cytotoxic effect of TNF (paragraph bridging pages 6 and 7). Under certain conditions it can also act as a carrier for TNF and thus maintain its prolonged beneficial effects (see page 6, lines 13-21, and Example 9, beginning at page 33).

This naturally occurring protein TBP-II, which was isolated from the urine, was found to include the following partial amino acid sequence: Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser- Thr in the portion of the protein sequenced by N-terminal sequence analysis (see page 7, lines 13-15).

The TBP-II encoded by the DNA of the present invention derived from human urine concentrate showed an apparent molecular weight of 30 kD in reducing SDS-PAGE analysis (page 7, lines 2-3).

The DNA of the present invention may also encode active fractions of TBP-II provided the fraction has the ability to inhibit the cytotoxic effect of TNF (see page 15, lines 11-17).

The present claims are drawn to the isolated DNA molecules which encode the newly discovered TBP-II protein and active fragments thereof as well as replicable expression vehicles

containing such DNA, host cells transformed with the replicable expression vehicle and processes for producing TBP-II by culturing such a transformant host cell (see page 8, lines 18-23, and claims 11-14 as originally filed).

One claim directed to the TBP-II protein was officially found to be allowable by the examiner in charge of the parent application. The claims drawn to the TBP-II protein in the parent application, 07/930,443, are now involved in an interference proceeding with the claims of U.S. patent 5,344,915.

THE PRIOR ART

The final rejection of January 30, 2001, contains no rejections over the prior art. Thus, there is no prior art which requires discussion in the present brief.

THE REJECTIONS

In the final rejection of January 30, 2001, claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention for the reasons elaborated in previous Office Actions. The reasons elaborated in previous Office Actions are believed to have been

restated in full in the Advisory Action of August 20, 1999, where the examiner stated:

> The specification does not provide adequate written description of the claimed invention. The legal standard for sufficiency of a patent's (or a specification's) written description is whether that description "reasonably conveys to the artisan that the inventor had possession at that time of the ... claimed subject matter", Vas-Cath, Inc. V. Mahurkar, 19 U.S.P.Q.2d 1111 (Fed. Cir. 1991). In the instant case, the specification does not convey to the artisan that the applicant had possession at the time of invention of the claimed DNAs and molecules containing said DNAs.
>
> The instant claims encompass an isolated DNA molecule or vectors or host cells which contain said DNA wherein said DNA encodes a protein consisting of naturally occurring TBP-II. There is no disclosure in the specification of an intact DNA sequence which encodes said molecule. There is no disclosure in the specification of any DNA sequence which encodes the claimed DNA. The claimed molecule recites physical features of a TBP-II protein and the amino acid sequences of a 10-13 amino acid sequence of the N terminal of a molecule that contains at least 250 amino acids. There is no disclosure in the specification of any DNA sequence which encodes the claimed molecule. In view of the aforementioned problems regarding description of the claimed invention, the specification does not provide an adequate written description of the invention claimed herein. See The Regents of the University of California v. Eli Lilly and Company, 43 USPQ2d 1398,1404-7 (Fed. Cir. 1997). In University of California v. Eli Lilly and Co., 39 U.S.P.Q.2d 1225 (Fed. Cir. 1995) the inventors claimed a genus of DNA species encoding insulin in different vertebrates or mammals, but had only described a single species of cDNA which encoded rat insulin.

The court held that only the nucleic acids [sic] species described in the specification (i.e. nucleic acids encoding rat insulin) met the description requirement and that the inventors were not entitled to a claim encompassing a genus of nucleic acids encoding insulin from other vertebrates, mammals or humans, id. at 1240. In the instant case, the specification has not provided even a single DNA sequence which encodes the claimed DNA. The Federal Circuit has held that if an inventor is "unable to envision the detailed constitution of a gene so as to distinguish it from other materials ... conception has not been achieved until reduction to practice has occurred", Amgen, Inc. v. Chugai Pharmaceutical Co, Ltd., 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991). Attention is also directed to the decision of The Regents of the University of California v. Eli Lilly and Company (CAFC, July 1997) wherein is stated: The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention. See In re Wilder, 736 F.2d 1516, 222 USPQ 369, 372-373 (Fed. Cir. 1984) (affirming rejection because the specification does "little more than outlin[e] goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate."). Accordingly, naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material.

Thus, as we have previously held, a cDNA is not defined or described by the mere name "cDNA," even if accompanied by the name of the protein that it encodes but requires a kind of specificity usually achieved by means of the recitation of the sequence of nucleotides that make up the cDNA. See Fiers, 984 F.2d at 1171, 25 USPQ2d at 1606.

Regarding applicants comments in the instant amendment about the criterion C(2) from the

interim written description guidelines, the following comments are made. The particular paragraph from C(2) which applicant quotes on page 14 of the instant amendment indicates that in order to meet the written description requirement the characteristics of the claimed invention need to be described "in such full, clear concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention". In the instant application, while applicant has disclosed information and methods to obtain the claimed nucleic acid sequence, applicant was clearly not in possession of the claimed invention at the time the instant application was filed. There is no disclosure in the specification of isolated nucleic acids encoding the molecule recited in the claims. Regarding the particular example from the interim written description guidelines which applicant quotes in page 15 of the instant amendment, said example differs from the instant application in that the example discloses a scenario wherein the applicant was in physical possession of the claimed molecule. In order to know that said molecule had the particular characteristics disclosed in said example, the molecule was isolated and demonstrated to have said characteristics. Therefore, applicant had physical possession of said molecule. It would be impossible to know the restriction and/or nuclease cleave sites without knowing the intact sequence of said nucleic acid or without having physically isolated the nucleic acid and empirically determined the information. In the case of the instant application, applicant has not demonstrated possession of the claimed invention because while applicant has disclosed a method for isolating said molecule, the molecule was not isolated. Similarly, regarding the enzyme example listed in page 16 of the instant amendment, in order to determine the various physical properties recited in said claim, it was necessary to have already obtained and possessed said molecule. In the case of the instant application, applicant has not

demonstrated possession of the claimed invention because while applicant has disclosed a method for isolating said molecule, the molecule was not isolated. Thus, the instant claims do not meet the criterion section C(2) from the interim written description guidelines. Regarding applicants theory that disclosure of a protein provides written description of the nucleic acid, there is no disclosure in the instant application of the amino acid sequence of TBP-II.

Regarding applicants comments in the instant amendment about University of California v. Eli Lilly, there is still no disclosure in the specification of any nucleic acid encoding the scope of the claimed invention (eg. a nucleic acid encoding TBP-II). There is also no disclosure in the specification of the amino acid sequence of intact TBP-II. While the specification discloses N-terminal amino acid sequence data indicating a possible partial amino acid sequence of 31 amino acids of TBP-II, said peptide contains at least 250 amino acids, wherein the identity of the vast majority of said amino acids has not been disclosed in the specification. In University of California v. Eli Lilly, the court held that only the nucleic acids [sic] species described in the specification (i.e. nucleic acids encoding rat insulin) met the description requirement and that the inventors were not entitled to a claim encompassing a genus of nucleic acids encoding insulin from other vertebrates, mammals or humans, id. at 1240. In the instant case, the specification has not provided even a single DNA sequence which encodes the claimed DNA. The Federal Circuit has held that if an inventor is "unable to envision the detailed constitution of a gene so as to distinguish it from other materials ... conception has not been achieved until reduction to practice has occurred", Amgen, Inc. v. Chugai Pharmaceutical Co, Ltd., 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991). Attention is also directed to the decision of The

Regents of the University of California v. Eli Lilly and Company (CAFC, July 1997) wherein is stated: The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention. See In re Wilder, 736 F.2d 1516, 222 USPQ 369, 372-373 (Fed. Cir. 1984) (affirming rejection because the specification does "little more than outlin[e] goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate."). Accordingly, naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material.

Regarding applicants comments that TBP-II protein is disclosed in the specification and the intact amino acid sequence of TBP-II could be obtained using the methods disclosed in the specification, this is not the issue under consideration. The Federal Circuit has held that if an inventor is "unable to envision the detailed constitution of a gene so as to distinguish it from other materials ... conception has not been achieved until reduction to practice has occurred", Amgen, Inc. v. Chugai Pharmaceutical Co, Ltd., 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991). Clearly, in the instant application, the inventor is unable to envision the detailed constitution of a nucleic acid so as to distinguish it from other materials because the sequence of the claimed nucleic acid was not known to the inventors at the time of the filing date of the instant application. Regarding applicants comments about the TBP-II protein, none of the claims of the instant invention are drawn to TBP-II protein. The claims under consideration are drawn to nucleic acids. The possession of an isolated protein in itself provides no written description of the identity of the nucleic acid encoding said protein in the absence of the complete amino acid sequence of said protein. Applicants response recites "Once the

complete amino acid sequence is known, all contiguous DNA sequences which encode such a protein are known in view of the known rules of the genetic code.". However, the complete amino acid sequence of TBP-II is not disclosed in the instant application. The instant application merely recites methods that could be potentially used to elucidate the nature of said sequence. In the absence of the disclosure of the claimed nucleic acid in the specification or the complete amino acid sequence of TBP-II there is no written description of the scope of the claimed invention. Regarding applicants comments that University of California v. Eli Lilly only applies to "genes" per se, this not stated in University of California v. Eli Lilly. In fact, in University of California v. Eli Lilly the court clearly states that:

An adequate written description of a DNA, such as the cDNA of the recombinant plasmids and microorganisms of the '525 patent, "requires a precise definition, such as by structure, formula, chemical name, or physical properties," not a mere wish or plan for obtaining the claimed chemical invention. Fiers v. Revel, 984 F.2d 1164, 1171, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993). Accordingly, "an adequate written description of a DNA requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself." Id. at 1170, 25 USPQ2d at 1606.

In the instant application, applicants has [sic] provided a plan and potential method for isolating the claimed nucleic acids, but have provided no written description of said nucleic acids.

In the final rejection of January 30, 2001, the examiner further stated with respect to applicants' arguments made subsequent to the Advisory Action of August 20, 1999:

Regarding applicants [sic] comments in the instant Brief, applicants comments are based on the syllogism enunciated in page 13 of said Brief. In said syllogism, applicant appears to argue that the DNA sequence encoding TBP II is an inherent property of the TBP II protein visa vie [sic] the inherent amino acid sequence of TBP II. However, the DNA sequence is not an inherent property of the TBP II protein because proteins do not encode nucleic acids. Regarding point 2 of said syllogism, while the amino acid sequence of TBP II is an inherent property of said protein, the nucleic acid sequence encoding said molecule is not an inherent property of the protein. There is no disclosure in the specification of the nucleic acid sequence of a DNA molecule encoding TBP II. The amino acid sequence of TBP II is not disclosed in the specification. While the amino acid sequence of TBP II is an inherent property, in order to determine the nucleic acid sequence based on said sequence, disclosure of said sequence is required as is the conversion of the amino acid sequence into appropriate nucleic acids encoding said protein. The amino acid sequence of TBP II was not known by applicant at the time of filing of the instant application, therefore, applicant was not in possession of the claimed nucleic acids. Furthermore, there is no disclosure in the specification of the nucleic acid sequence of a DNA encoding TBP II. It is also clear that a DNA sequence encoding a protein is not an inherent property of a protein. Applicants [sic] syllogism puts forth an argument as to why the DNA sequence encoding TBP II would be obvious based on the inherent amino acid of TBP II. In view of the fact that there is no literal description of a nucleic acid sequence encoding a DNA sequence in the specification, applicants [sic] syllogism would at best explain why the claimed nucleic acid is obvious in view of the inherent amino acid sequence of the TBP II protein. However, obviousness is not the appropriate standard with regards to issues of written

description. The CAFC stated in Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1997) that:

3. Patentability/Validity -- Specification -- Written description (§115.1103)

Patent's entitlement to earlier filing date extends only to that which is disclosed in prior application, and does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed; one shows that one is "in possession" of invention of patent by describing invention, with all its claimed limitations, not that which makes it obvious, and although prior application need not describe claimed subject matter in exactly same terms used in claims, prior specification must contain equivalent description of claimed subject matter, and description which renders obvious invention for which earlier filing date is sought is not sufficient.

The CAFC also stated in Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1977) that:

The invention is, for purposes of the 'written description' inquiry, whatever is now claimed.") (emphasis in original). One does that by such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention. Although the exact terms need not be used in haec verba, see Eiselstein v. Frank, 52 F.3d 1035, 1038, 34 USPQ2d 1467, 1470 (Fed. Cir. 1995) ("[T]he prior application need not describe the claimed subject matter in exactly the same terms as used in the claims ...."), the specification must contain an equivalent description of the claimed subject matter. A description which renders obvious the invention for which an earlier filing date is sought is not sufficient.

> There is no disclosure in the specification of an isolated nucleic acid encoding TBP II or the nucleic acid sequence encoding said molecule. Therefore, at the timing [sic] of filing applicant was not in possession of the claimed invention. While applicants [sic] syllogism establishes why the claimed nucleic acid would be obvious based on the inherent amino acid sequence of TBP II, obviousness is not the appropriate standard for written description. Regarding claims 35-38, 43, 44, 46-49, 51-54, 56-61, 63, 64 there is no disclosure in the specification of nucleic acids encoding the fragments of TBP II recited in the claims. Applicants [sic] syllogism does not disclose the identity of said fragments or even render the identity of said fragments obvious.

Claims 35-38, 43, 44, 46-49 and 51 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention, the examiner stating:

> There is no support in the specification as originally filed for the claimed DNA molecules encoding the fragment of claim 35, part (2) or claim 36, part (2) or claim 46 part (2) or claim 51, part (2). Regarding original claim 11, said claim is drawn to a DNA molecule encoding TBP II, not a fragment thereof. There is no disclosure in the specification as originally filed of DNA molecules encoding the aforementioned fragments recited in the claims. There is no written description of the scope of the claimed inventions in the specification as originally filed (the claimed inventions constitute new matter).

Regarding applicants [sic] comments, the specification page 15, lines 11-17 refers to protein molecules, not nucleic acid molecules. Regarding the specification, page 4, said passage refers to nucleic acids encoding proteins, not fragments of a protein with a particular functional activity. Regarding the specification, page 7, said passage of the specification refers to proteins and also does not specify that "proteins substantially homologous" refers to protein fragments with a particular functional activity. Regarding the specification, page 9, lines 13-21, said passage refers to oligonucleotides used as probes to detect the DNA encoding TBP-II. This is not the claimed invention. The specification page 16 also does not disclose the scope of the claimed invention (eg. a nucleic acid encoding a fragment with the functional activity recited in the claims. Applicant appears to be arguing that the instant limitation is obvious in view of the disclosure of the specification. However, obviousness is not the appropriate standard with regards to issues of written description. The CAFC stated in Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1997) that:

3. Patentability/Validity -- Specification -- Written description (§115.1103)

Patent's entitlement to earlier filing date extends only to that which is disclosed in prior application, and does not extend to subject matter which is not disclosed, but would be obvious over what is expressly disclosed; one shows that one is "in possession" of invention of patent by describing invention, with all its claimed limitations, not that which makes it obvious, and although prior application need not describe claimed subject matter in exactly same terms used in claims, prior specification must contain equivalent description of claimed subject matter, and description which renders obvious invention

> *for which earlier filing date is sought is not sufficient.*
>
> The CAFC also stated in Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed. Cir. 1977) that:
>
> *The invention is, for purposes of the 'written description' inquiry, whatever is now claimed.") (emphasis in original). One does that by such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention. Although the exact terms need not be used in haec verba, see Eiselstein v. Frank, 52 F.3d 1035, 1038, 34 USPQ2d 1467, 1470 (Fed. Cir. 1995) ("[T]he prior application need not describe the claimed subject matter in exactly the same terms as used in the claims ...."), the specification must contain an equivalent description of the claimed subject matter. A description which renders obvious the invention for which an earlier filing date is sought is not sufficient.*

Claim 63 has been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention, the examiner stating:

> There is no support in the specification as originally filed for the claimed DNA molecules. The oligonucleotides disclosed in pages 9-11 of the specification are complementary to the DNA encoding TBP-II. Therefore, they do not encode TBP-II, because they are antisense to the TBP-II molecule. The instant claim recites oligonucleotides that encode DNA encoding TBP-II. There is no

disclosure of such an invention in the specification as originally filed.

ISSUES

The following issues are presented in this appeal:

1. Is there adequate written description for a claim covering all DNA sequences which encode a novel isolated protein defined by a partial amino acid sequence and other characterizing features?

2. Is there adequate written description for the claimed DNA molecules encoding a fragment of TBP-II that has the ability to inhibit the cytotoxic effect of TNF?

3. Is there adequate written description for the claimed DNA molecules encoding a fragment of TBP-II having a sufficient length to serve as an oligonucleotide probe?

GROUPING OF CLAIMS

For each rejection, all of the claims grouped for that rejection stand or fall together.

A R G U M E N T

The Specification as Filed Describes the Claimed DNA in Sufficient Detail that One Skilled in the Art Can Reasonably Conclude that the Inventor Had Possession of the Claimed DNA

Applicant's position with respect to the written description rejection can be thumbnailed by the following syllogism.

1. The complete amino acid sequence of a protein is an inherent property of an isolated protein which has been fully characterized by partial amino acid sequence and other characteristics.

2. The complete amino acid sequence automatically puts one in possession of all DNA sequences encoding it, as the genetic code is based on an unequivocal correspondence between amino acids and encoding DNA codons.

3. Therefore, one who has isolated a novel protein and fully characaterized it by partial amino acid sequence and other characteristics, is inherently in possession of the complete amino acid sequence thereof and, thus, also inherently in possession of all DNA sequences encoding that amino acid sequence.

4. The present specification discloses the isolation of the TBP-II protein and sets forth sufficient identifying characteristics, including partial amino acid sequence, to establish that the inventors were in possession of that protein, thereby establishing an adequate written description for the TBP-II protein.

5. As applicants have demonstrated possession of the TBP-II protein, applicants were also necessarily in possession of its inherent amino acid sequence as well as all of the DNA sequences encoding that amino acid sequence.

With respect to the first paragraph of the above syllogism, reference is made to Ex parte Yamaguchi, 6 USPQ2d 1805, 1807 (BdPatApp&Int 1987), where it states:

> Moreover, it is well settled that from a standpoint of patent law, a compound and all of its properties are inseparable. They are one and the same. *In re Papesch* 50, CCPA 1084, 315 F2d 381, 137 USPQ 43 (1963). In our view, the X-ray diffraction spectrum, like the graphic formulae, the chemical nomenclature, etc., is merely a symbol by which the compounds can be identified, classified and compared.

The same is true for the amino acid sequence of a protein.

See also Ex parte Marsili, 214 USPQ 904 (PTOBdApp 1979) which held that a change in the structural formula of a chemical compound that was adequately described in terms of its characteristics in the original specification did not violate the description requirement. Furthermore, in the Board decision of Ex parte Deuel, 27 USPQ2d 1360, 1363 (BdPatApp&Int 1993), the Board noted the examiner's position that the amino acid sequence is an inherent characteristic of the protein.

Moreover, this point has been conceded by the examiner in the final rejection of January 30, 2001, at the bottom of page 3, where he twice uses the phrase "while the amino acid sequence of TBP II is an inherent property of said protein ...".

In this case, not only is the amino acid sequence an inherent property of TBP-II, but, in light of the partial amino acid sequence of the protein and the other characterizing

features disclosed, as well as the method for obtaining the protein, one of ordinary skill in the art would obtain the entire amino acid sequence of the protein without undue experimentation.

As to the second paragraph of the syllogism, it is an undisputed scientific fact that given the complete amino acid sequence of a protein, coupled with knowledge of the genetic code, one is in possession of the genus of all of the DNA sequences which will encode that complete amino acid sequence. The genetic code provides an unequivocal direct relationship of amino acid sequences and associated nucleic acid codons. In In re Deuel, 34 USPQ2d 1210, 1216 (Fed. Cir. 1995), the court noted that, with the aid of a computer, a person of ordinary skill in the art may be able to identify all members of the claimed genus of DNA sequences which encode a complete amino acid sequence.

Reference is also made to footnote 14 of the Guidelines for Examination of Patent Applications Under the 35 U.S.C. §112, paragraph 1, "Written Description" Requirement (Federal Register, 66:1099-1111 (2001) at 1108) (hereinafter "Written Description Guidelines"), which explicitly states:

> [a] genetic code table would correlate a known amino acid sequence with a genus of coding nucleic acids ... .

Thus, if one is in possession of the complete amino acid sequence encoded by a claimed DNA sequence, then one is necessarily in possession of the entire claimed DNA genus.

The conclusion in paragraph three of the above syllogism follows naturally from the established facts of the first two paragraphs. The complete amino acid sequence of a protein is an inherent property of an isolated and fully characterized protein. Furthermore, the complete amino acid sequence automatically puts one in possession of all DNA sequences encoding it. Thus, it must follow that one who is possession of an isolated protein and has fully characterized it by partial amino acid sequence and other characteristics, is inherently in possession of all DNA sequences encoding the complete amino acid sequence of that protein.

As to the fourth paragraph of the above syllogism, it is not believed that the examiner disputes the fact that there is written description for the TBP protein in the application as originally filed. Indeed, it should be noted that during the prosecution of this case the examiner has not refuted this particular part of the syllogism. In the Advisory Action of August 20, 1999, beginning at the second full paragraph of page 5, the examiner stated:

> Regarding applicants [sic] comments that TBP-II protein is disclosed in the specification and the intact amino acid sequence of TBP-II could be obtained using the method disclosed

> in the specification, this is not the issue under consideration. ... Regarding applicants [sic] comments about the TBP-II protein, none of the claims of the instant invention are drawn to TBP-II protein.

Thus, the examiner carefully avoids the issue. The examiner cannot take the position that the fourth paragraph of the syllogism as written hereinabove is incorrect because TBP protein claims have been found to be allowable in the parent application 07/930,443.

Furthermore, the fourth paragraph of the syllogism is consistent with the Written Description Guidelines. Section II.A.3.a of these Guidelines states that, for original claims, one must first determine whether there is sufficient written description to inform a skilled artisan that applicant was in possession of the claimed invention as a whole at the time the application was filed. Possession may be shown in many ways. One way is by describing an actual reduction to practice of the claimed invention. This section also specifically states:

> An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention.

Here, with respect to the protein, there was an actual reduction to practice as the protein was actually isolated. Furthermore, the protein was described by a partial amino acid sequence and, by other relevant identifying characteristics, such as by a number of methods of production

and by the biological functions thereof, all of which characteristics are sufficient to permit a person skilled in the art to recognize that the inventor had possession of the claimed invention. The fact that the protein was adequately described to comply with the written description requirement is evidenced by the fact that at least one protein claim was allowed during the prosecution of the parent application 07/930,443, which was involved in an interference proceeding with the claims of U.S. patent 5,344,915, and is now involved in an appeal of a Board decision favorable to applicants.

The conclusion in the fifth paragraph of the syllogism also follows naturally from the established facts and conclusions of the previous paragraphs.

The conclusion reached in the above syllogism is consistent with the Written Description Guidelines, which were published on January 5, 2001. These guidelines, in the second paragraph of Section I (FR 66:1104) state:

> To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.

Similarly, the last paragraph of Section I (at 1105) reads:

> The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as

> of the filing date sought, applicant was in possession of the invention as now claimed.

Thus, it is possession of the claimed invention which is important. The above syllogism establishes that applicant was in possession of the genus of DNA sequences that encode the single species of naturally occurring human TBP-II. As applicant was in possession of the invention as now claimed, the fundamental factual inquiry necessary to satisfy the written description requirement must be answered in the affirmative.

There is nothing in the case law cited by the examiner which precludes an applicant from claiming the genus of DNA which encodes an adequately disclosed protein. Admittedly, if the present claims were directed to the human cDNA encoding TBP-II, the case law would require a 35 USC 112, first paragraph, written description rejection because it would have been impossible for applicant to envision that single specific sequence which is the cDNA. Thus, even though there is adequate written description in the present specification for the genus of all DNA sequences which encode a given amino acid sequence, there is admittedly no written description for the specific species of the cDNA, and indeed the specific species of the cDNA is not being specifically claimed in the present application. In the Eli Lilly case relied upon by the

examiner, discussed in detail hereinbelow, the claims being reviewed for compliance with the written description requirement were directed to the cDNA and not to broad DNA claims covering any DNA sequence which encodes the novel protein. Indeed, in that case, the protein was not novel and therefore a generic DNA claim, such as is presently claimed, would have been obvious.

More specifically, the examiner relies mainly on University of California v. Eli Lilly and Co., 43 USPQ2d 1398 (Fed. Cir. 1997). While that case relates to the infringement of two patents, i.e., patents 4,652,525 and 4,431,740 owned by the Regents of the University of California (UC), validity issues relating to the written description requirement of the first paragraph of 35 USC 112 were raised only with respect to the '525 patent. Copies of the front page and claims of these two patents are attached hereto as Appendices B and C. It can be seen that, in the '525 patent, all of the claims are directed to insulin-encoding cDNA, or the reverse transcript of mRNA which encodes insulin, which is synonymous with cDNA. Note that the Federal Circuit in the Lilly case at page 1405 characterizes claims 1 and 2 of the '525 patent as being claims "which *generically* recite cDNA encoding vertebrate insulin, and claim 4, which is directed *generically* to cDNA encoding mammalian insulin" [emphasis original] and that dependent

claims 6 and 7 "similarly recite cDNA encoding vertebrate insulin." As to claim 5, the court stated, at pages 1404-1405:

> Claim 5 is directed to a recombinant prokaryotic microorganism modified so that it contains "a nucleotide sequence having the structure of the reverse transcript of an mRNA of a [human], which mRNA encodes insulin."

Thus, the definition of the claimed microorganism is one that requires human insulin-encoding cDNA. The validity of claim 3 was not before the court. Thus, it is very clear that all of the claims being construed for compliance with the written description requirement were claims directed to cDNA, i.e., the naturally occurring sequence which is only one of the myriad of possible sequences which encode human insulin due to the degeneracy of the genetic code. Therefore, the holdings in the Lilly case which require that the sequence of the cDNA be known before that cDNA can be in the possession of the inventors so as to satisfy the written description requirement, are all related to the specific situation before the court in which all that is being claimed is cDNAs, either a cDNA of a single species or a genus of cDNAs of a plurality of animal species.

In the Advisory Action of August 20, 1999, in response to applicant's previous arguments that the Lilly case applied only to cDNAs *per se*, the examiner refers to page 1404 of Lilly where it states:

> An adequate written description of a DNA, such as the cDNA of the recombinant plasmids and microorganisms of the '525 patent, "requires a precise definition, such as by structure, formula, chemical name, or physical properties," not a mere wish or plan for obtaining the claimed chemical invention.

However, when the DNA claim is written broadly so as to include all DNA which encodes a particular amino acid sequence, the description of the amino acid sequence or sufficient characterizing information to establish that applicant was in possession of a novel protein, is sufficient to satisfy the requirement for a precise definition. Indeed, the examiner himself stated at page 6 of the Advisory Action:

> In the absence of the disclosure of the claimed nucleic acid in the specification, or the complete amino acid sequence of TBP-II there is no written description of the scope of the claimed invention. [Emphasis added]

Thus, the examiner appears to admit that if applicant were in possession of the complete amino acid sequence of TBP-II, then applicant would automatically be in possession of the claimed nucleic acid sequence which anyone of ordinary skill in the art could write as a formula once the complete amino acid sequence is known. Indeed, reference is made to claim 5 of the '740 patent involved in the Lilly case in which such a DNA sequence broad enough to encompass all DNAs which encode human proinsulin is set forth. Such a formula can readily be

prepared for any given amino acid sequence without any knowledge of the naturally occurring cDNA.

Here, applicant readily admits that the specification does not contain a complete amino acid sequence of TBP-II. However, it does disclose a partial amino acid sequence and sufficient other characterizing features to establish that applicant was in possession of the protein. Indeed, applicant had isolated the protein. The written description requirement was satisfied for the protein as is evidenced by the allowability of at least one protein claim in the parent application. As the complete amino acid sequence of a protein is an inherent property of an adequately described protein which is in possession of the applicant (see the examiner's admission to this effect in the last paragraph of page 3 of the Final Rejection of January 30, 2001) and a genetic code table can correlate any amino acid sequence with a genus of coding nucleic acids (see above-quoted portion of the Written Description Guidelines), it must necessarily follow that adequate written description of a protein is inherently an adequate written description of a broad DNA claim which encompasses all nucleotide sequences which encode that protein.

There is nothing in the Written Description Guidelines that mandates a rejection of the present claims under the written description requirement. Indeed, in the response to comment 7 at

page 1101 of the Federal Register notice, the material accompanying the Written Description Guidelines makes clear that they do not impose a *per se* requirement for reduction to practice in any technology to satisfy the written description requirement. In footnote 53 (FR 66 at 1110), the Guidelines discuss a case where actual reduction to practice was required to establish conception of the invention, but concludes:

> In such instances, the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor's idea of the invention.

Here, while applicant may not have reduced to practice a specific DNA, applicant has reduced to practice the protein. Applicant has possession of the protein and has provided adequate written description of the protein. The complete amino acid sequence of the protein is an inherent property of the protein. Because the formula of all DNA which encompass that amino acid sequence is dictated by the genetic code, i.e., is a fixed formula, the DNA sequence is as much an inherent property of the adequately described protein which has been reduced to practice as is the complete amino acid sequence thereof. Therefore, there is no actual uncertainty that undermines the specificity of the inventor's idea of the invention, such as would require an actual reduction to

practice of a DNA before an applicant can be in possession thereof.

The statement in Section II.A.3.a. of the Written Description Guidelines (66 FR at 1206) is also applicable where it states:

> An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention, *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. [footnotes omitted]

Such sufficiently detailed, relevant identifying characteristics have been provided in the present specification for the protein. As the complete amino acid sequence of the protein is an inherent characteristic of the protein and as the formula for DNA which encodes the complete amino acid sequence is a fixed formula determined by the genetic code, such DNA formula is also an inherent characteristic of the adequately described protein.

Furthermore, the claims effectively include a partial nucleic acid sequence. All of the present claims recite at least 10 amino acid residues of the protein encoded by the DNA. Thus, at least 30 nucleotides of the DNA are disclosed. Regardless of the fact that the DNA molecule of the present

invention is much longer than 30 nucleotides, this is an important unique bit of characterizing information. This piece of nucleotide structure, in conjunction with the characterizing information that the DNA encodes a protein having the ability to inhibit the cytotoxic effects of TNF, provides sufficient relevant identifying characteristics to comply with the criteria of the above-quoted portion of the Revised Interim Guidelines.

The same paragraph of the Written Description Guidelines goes on to state:

> If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.

This sentence further supports the conclusion reached by the syllogism set forth hereinabove. Accordingly, for the reasons discussed in detail hereinabove, possession of a novel protein and a written description thereof sufficient to comply with the written description requirement of the first paragraph of 35 U.S.C. §112 inherently places one in possession of the formula of all DNA which encodes that protein. As the complete amino acid sequence of that protein is an inherent property of the protein and the generic DNA sequence which encodes that amino acid sequence is directly correlatable therewith by means of a

genetic code table, a holding that the present claims comply with the written description requirement would be fully consistent with the Written Description Guidelines.

In the final rejection of January 30, 2001, the examiner stated:

> [T]he DNA sequence is not an inherent property of the TBP-II protein because proteins do not encode nucleic acids.

While this statement may be literally true, it is not relevant because the TBP-II protein is adequately described by partial amino acid sequence and other characterizing properties, and is encoded by nucleic acids. The genetic code provides a direct relationship of amino acid sequences and associated nucleic acid codons. Thus, the generic set of DNA sequences which encode the complete amino acid sequence of TBP-II is as much an inherent property of the TBP-II protein as is that amino acid sequence itself.

The examiner states that applicants' syllogism puts forth an argument as to why the DNA sequence encoding TBP-II would be obvious based on the inherent amino acid sequence of TBP-II, but obviousness is not the appropriate standard with regard to issues of written description. Indeed, at page 5 of the Final Rejection of January 30, 2001, the examiner concedes this obviousness, where he states:

> While applicants [sic] syllogism establishes why the claimed nucleic acid would be obvious based on the inherent amino acid sequence of TBP-II, obviousness is not the appropriate standard for written description.

However, the examiner's reliance on cases about obviousness and written description is misplaced. Applicants are not here trying to establish possession of something never mentioned in the specification, or a variant of what was disclosed, because it might be obvious from what was mentioned in the specification. Here, the present specification is quite explicit that the DNA encoding TBP-II is part of the present invention. See the last paragraph of page 4 of the specification, as well as the disclosure at page 8, line 18, through page 14, line 8. Note also that claim 11, as originally filed, was specifically directed to a DNA molecule comprising the nucleotide sequence coding for the TBP-II of claim 1.

The case of Lockwood v. American Airlines Inc., 41 USPQ2d 1961 (Fed Cir 1997), cited by the examiner, poses a different question. Note where it states at 1966:

> The question is not whether a claimed compound is an obvious variant of that which is disclosed in the specification. Rather, a prior application itself must describe an invention, and do so in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed invention as of the filing date sought.

Here, the DNA sequence is not a "variant" of that which is disclosed. Claim 11, as originally filed, clearly describes the DNA encoding TBP-II. The issue here is whether the specification sufficiently establishes that applicants were in possession of that originally-claimed DNA. In this regard, reference is made to the Written Description Guidelines at Section II.A.3.a., which relates to whether there is sufficient written description to support original claims. There it states:

> An adequate written description of the invention may be shown by any description of sufficient relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention.

As the examiner admits that applicants' syllogism establishes why the claimed nucleic acid would be obvious based on the inherent amino acid sequence of TBP-II, this sufficiently answers the question of whether those of ordinary skill in the art would recognize that the inventors had possession of the claimed invention at the time that it was filed. Furthermore, inherency is not a concept of obviousness. When the issue of inherency is raised with respect to the disclosure of a prior art reference, the reference is considered to anticipate if the feature is inherent, not establish the obviousness of that feature. Thus, the examiner's concession that the syllogism establishes why the claimed nucleic acid would be obvious based

on the inherent amino acid sequence of TBP-II, is effectively a concession that that nucleic acid sequence is inherent (as he must in view of the genetic code), thereby establishing that applicants had possession of the claimed invention.

Reversal of the examiner and withdrawal of this rejection are therefore respectfully urged.

The Specification as Filed Adequately Describes the Claimed DNA Molecules Encoding a Fragment of TBP-II that Has the Ability to Inhibit the Cytotoxic Effect of TNF

The rejected claims, such as claim 51, encompass in paragraph (2) thereof isolated DNA molecules comprising "a contiguous nucleotide sequence coding for a fragment of said TBP-II which has the ability to inhibit the cytotoxic effect of TNF." It is the examiner's position that the specification does not demonstrate that the present inventors were in possession of such DNA encoding fragments of TBP-II at the time the invention was made. However, it can be demonstrated that the reference to DNA encoding active fragments of TBP-II is sufficiently supported in the present specification to establish that the inventors were in possession of the invention at the time that the application was filed and, therefore, in full compliance with the written description requirement of the first paragraph of 35 U.S.C. §112. As stated in Nelson v. Bowler, 1 USPQ2d 2076, 2078 (BdPatApp&Int 1986):

> It is not necessary that the claimed subject matter be described in *ipsis verbis* to satisfy the written description requirement of 35 USC 112.

It is sufficient that the specification "conveys clearly to those skilled in the art, to whom it is addressed, in any way, the information that the applicant has invented the subject matter later claimed." (In re Wertheim 191 USPQ 90, 97 (CCPA 1976)). Adequate disclosure may be by any means, since the objective is to communicate the invention to the reader who is skilled in the art. See Standard Oil Co. v. Montedison, S.p.A., 212 USPQ 327, 337-8 (3d Cir. 1981).

Here, the specification as a whole makes it clear to one skilled in the art that the applicants were in possession of the claimed subject matter at the time of filing of the application. It is clear that the specification describes active fractions of the TBP-II protein. Such active fractions are described and defined on page 15, lines 11-17. Further, it is clear that the specification contains written description for DNA sequences "coding for TBP-II or for a protein substantially homologous therewith" (see page 4, lines 16-17). While the term "proteins substantially homologous therewith" is not specifically defined in the specification, those of ordinary skill in the art would expect that those modifications of the TBP protein described in the specification would be considered to be proteins substantially homologous therewith.

In this regard, reference is made to page 7, lines 18-22, which states:

> The present invention encompasses a protein comprising the above sequence, herein referred to as TBP-II, as well as any other polypeptide in which one or more amino acids in the structure of natural TBP-II are deleted or replaced with other amino acids, or one or more amino acids are added thereto, as long as they have human TBP-II activity.

Those of ordinary skill in the art would understand this to be a description of homologs of TBP-II. This description includes other polypeptides in which one or more amino acids in the structure of natural TBP-II are deleted, as long as they have human TBP-II activity, in other words, active fragments. It is, thus, apparent that such active fragments are understood to be included in the phrase "proteins substantially homologous therewith" and, therefore, there is written description of DNA encoding active fragments of TBP-II.

Furthermore, the specification in several areas specifically refers to DNA encoding fragments of TBP-II, although not necessarily the active fragments described on page 15.

On page 9, lines 13-21, there is a description of a synthetic oligonucleotide whose sequence is derived from the amino acid sequence of a fragment of the protein. Note also page 10, lines 3-5, which states:

> The invention also relates to synthetic oligonucleotides to be used as probes to the DNA coding for TBP-II. They are synthesized by known methods on the basis of the amino acid sequence of fragments of TBP-II.

Another disclosure of DNA encoding fragments of TBP-II appears in the last three lines of page 16, which refer to the fusion of one of the possible nucleotide sequences coding for a fragment of TBP-II to a gene coding for Protein A. While these descriptions of DNA encoding fragments of TBP-II are in a different context from DNA encoding active fragments, it is clear from a consideration of the specification as a whole that, at the time the invention was made, applicants were in possession of DNA encoding fragments of TBP-II; applicants were in possession of active fragments of TBP-II; and applicants were in possession of DNA encoding TBP-II and proteins substantially homologous therewith. The aggregate of these disclosures should be sufficient to establish that the applicants invented DNA encoding active fragments of TBP-II, i.e., that applicants were in possession of the claimed DNA encoding active fragments of TBP-II at the time of the filing of the application. To refuse to permit applicants to claim DNA encoding active fragments of the protein despite a disclosure of the entire protein and active fragments thereof, a disclosure of DNA encoding the protein and proteins substantially homologous thereto, and disclosure of DNA

encoding other fragments of the protein, would be a hypertechnical application of the written description requirement, exalting form over substance.

As stated at MPEP §2163:

> Under *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 111, 117 (Fed Cir 1991), to satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed. The test for sufficiency of support in a parent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." [emphasis added]

It is, thus, clear that there is a requirement for reasonableness. The conveyance must be with reasonable clarity and the specification must reasonably convey that applicant was in possession of the invention. For the reasons discussed above, the present disclosure reasonably conveys this fact with reasonable clarity.

In the Final Rejection, the examiner states that the above-quoted portion of the specification at page 17, lines 18-22, refers to "proteins" and does not specify that "proteins substantially homologous" refers to protein fragments with a particular functional activity. However, it can be seen from the quotation that the TBP-II protein encompasses "any other

polypeptide", including polypeptides formed upon deletion of amino acids from TBP-II. It would be reasonable for one of ordinary skill in the art to consider this definition when trying to interpret the term "a protein substantially homologous therewith" in claim 11 as originally filed. The term "protein" is usually defined broadly as "a macromolecule made up of one or more chains of amino acids joined covalently through peptide bonds."[1] An active fragment of TBP-II is a "protein" by this definition.

The examiner also states that applicants appear to be arguing that the instant limitation is obvious in view of the disclosure of the specification. However, this is not what applicants are arguing. Applicants are arguing that the specification demonstrates that applicants were in possession of DNA encoding active fragments of TBP-II as proper construction of the specification would establish that the DNA encoding "a protein homolog" of TBP-II encompasses the active fragments thereof which are defined in the specification. References to the other portions of the specification are merely to show that this is a reasonable interpretation of the

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[1] Coombs, Dictionary of Biotechnology, 2nd Ed., MacMillan Press Ltd., London (1994), first sentence of definition of "protein". See also Stedman's Medical Dictionary, 26th Ed., Williams & Wilkins, Baltimore, MD (1995), p. 1442, in which the first sentence of the definition of "protein" reads:

> Macromolecules consisting of long sequences of α-amino acids [H_2N-CHR-COOH] in peptide (amide) linkage (elimination of H_2O between the α-NH_2 and α-COOH of successive residues).

specification, not merely that DNA encoding such fragments would be obvious merely from the disclosure of protein fragments.

Accordingly, reversal of the examiner and withdrawal of this rejection is also respectfully urged.

The Specification as Filed Describes the Claimed DNA Molecules Encoding a Fragment of TBP-II Having a Sufficient Length to Serve as an Oligonucleotide Probe

Claim 63 is directed to an isolated DNA molecule comprising a sequence encoding a fraction of TBP-II, which sequence has a sufficient length to serve as an oligonucleotide probe. This claim is supported by the specification at page 9, line 13, through page 11, line 10. This portion of the specification clearly provides written description for DNA encoding synthetic oligonucleotide probes whose sequence is derived from the amino acid sequence of a fragment of TBP-II. It is the examiner's position that oligonucleotide probes must be complementary to the DNA encoding TBP-II and, therefore, cannot encode a fragment of TBP-II because they are antisense to the TBP-II molecule. The examiner states that there is no disclosure of such an invention in the specification as originally filed. It is respectfully urged, however, that this is not correct.

The cDNA or the genomic DNA coding for TBP-II is double-stranded. Oligonucleotide probes need not be

complementary to the sense strand of this double-stranded DNA. Those of ordinary skill in the art understand that such oligonucleotide probes may hybridize to either the sense or antisense strand of the double-stranded DNA. Thus, such probes having the same sequence as the sense strand of the DNA will work equally as well as those having the same sequence as the antisense strand as such a probe it will hybridize to the antisense strand of the double-stranded DNA which is being probed. Note, for example, page 10, lines 20-25, of the present specification, where it states:

> However, only one member of the set contains the nucleotide sequence that is identical to the nucleotide sequence of the gene. Its presence within the set and its capability to hybridize to DNA even in the presence of the other members of the set, makes it possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the peptide. [emphasis added]

This clearly suggests that the nucleotide sequence of the probe may be identical to the nucleotide sequence of the gene. It will still bind to the gene because the gene is double-stranded.

Accordingly, those of ordinary skill in the art will understand that the present inventors were in possession of the isolated DNA molecules as defined in claim 63, which molecules actually encode a fraction of the naturally-occurring TBP-II.

Reversal of the examiner and withdrawal of this rejection is, therefore, also respectfully urged.

CONCLUSION

The claims as submitted are believed to truly set forth the inventive concept of the present invention and to fully comply with the written description requirement of the first paragraph of 35 U.S.C. §112. Accordingly, reversal of the examiner and allowance of claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64 are earnestly solicited.

Respectfully submitted,

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APPENDIX A

11. An isolated DNA molecule comprising a contiguous nucleotide sequence coding for a protein consisting of naturally occurring human Tumor Necrosis Factor (TNF) Binding Protein II, herein designated TBP-II, said TBP-II including the amino acid sequence: Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis, said protein having the ability to inhibit the cytotoxic effect of TNF, wherein said naturally occurring TBP-II protein is the same as that protein having the ability to inhibit the cytotoxic effect of TNF which, after being purified by subjecting a crude protein recovered from a dialyzed concentrate of human urine to affinity chromatography on a column of immobilized TNF, elutes from a reversed-phase high pressure liquid chromatography column as a single peak in a fraction corresponding to about 31% acetonitrile and shows a molecular weight of about 30 kDa when measured by SDS-PAGE under reducing conditions.

12. A replicable expression vehicle comprising the DNA molecule of claim 11 and capable, in a transformant host cell, of expressing said protein.

13. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 12.

35. An isolated DNA molecule in accordance with claim 51, comprising

(1) the nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

i. includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

ii. the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells, or

(2) a contiguous nucleotide sequence coding for a fragment of said TBP-II which has the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells.

36. An isolated DNA molecule comprising

(1) the nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

i. includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

ii. the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells; and

iii. a molecular weight of about 30kd in reducing SDS-PAGE analysis, or

(2) a contiguous nucleotide sequence coding for a fragment of said TBP-II which has the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells.

37. A replicable expression vehicle comprising the DNA molecule of claim 51 and capable, in a transformant host cell, of expressing said protein.

38. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 37.

43. A replicable expression vehicle comprising the DNA molecule of claim 35 and capable, in a transformant host cell, of expressing said protein.

44. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 43.

46. An isolated DNA molecule comprising (1) a contiguous nucleotide sequence coding for a protein consisting of naturally occurring human Tumor Necrosis Factor (TNF) Binding Protein II, herein designated TBP-II, said TBP-II including the amino acid sequence: Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis, said protein having the ability to inhibit the cytotoxic effect of TNF, wherein said naturally occurring TBP-II protein is the same as that protein having the

ability to inhibit the cytotoxic effect of TNF which, after being purified by subjecting a crude protein recovered from a dialyzed concentrate of human urine to affinity chromatography on a column of immobilized TNF, elutes from a reversed-phase high pressure liquid chromatography column as a single peak in a fraction corresponding to about 31% acetonitrile and shows a molecular weight of about 30 kDa when measured by SDS-PAGE under reducing conditions, or (2) a contiguous nucleotide sequence coding for a fragment of said TBP-II which has the ability to inhibit the cytotoxic effect of TNF.

47. An isolated DNA molecule in accordance with claim 51, wherein said nucleotide sequence is the sequence of (2).

48. A replicable expression vehicle comprising the DNA molecule of claim 47 and capable, in a transformant host cell, of expressing said protein.

49. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 48.

51. An isolated DNA molecule comprising

(1) a contiguous nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

(a) includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

(b) has the ability to inhibit the cytotoxic effect of TNF; or

(2) a contiguous nucleotide sequence coding for a fragment of said TBP-II which has the ability to inhibit the cytotoxic effect of TNF.

52. An isolated DNA molecule comprising a contiguous nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

(a) includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

(b) has the ability to inhibit the cytotoxic effect of TNF.

53. A replicable expression vehicle comprising the DNA molecule of claim 52 and capable, in a transformant host cell, of expressing said protein.

54. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 53.

ii. the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells; and

iii. a molecular weight of about 30kd in reducing SDS-PAGE analysis.

60. A replicable expression vehicle comprising the DNA molecule of claim 59 and capable, in a transformant host cell, of expressing said protein.

61. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 60.

63. An isolated DNA molecule comprising a sequence encoding a fraction of a naturally-occurring human tumor necrosis factor (TNF) binding protein (TBP-II) having the following the following characteristics:

(a) includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

(b) has the ability to inhibit the cytotoxic effect of TNF;

said sequence having a sufficient length to serve as an oligonucleotide probe.

64. An isolated DNA molecule comprising a sequence encoding a fraction of a naturally-occurring human tumor

necrosis factor (TNF) binding protein (TBP-II) having the following the following characteristics:

(a) includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

(b) has the ability to inhibit the cytotoxic effect of TNF;

said sequence being of a sufficient length to encode a fragment of TBP-II of sufficient length to serve as an immunogen for raising antibodies against TBP-II when fused to protein A.

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APPENDIX C

United States Patent [19]

Bell et al.

[11] **4,431,740**

[45] **Feb. 14, 1984**

[54] **DNA TRANSFER VECTOR AND TRANSFORMED MICROORGANISM CONTAINING HUMAN PROINSULIN AND PRE-PROINSULIN GENES**

[75] Inventors: **Graeme Bell; Raymond Pictet; Howard M. Goodman; William J. Rutter, all of San Francisco, Calif.**

[73] Assignee: **The Regents of the University of California, Berkeley, Calif.**

[21] Appl. No.: **386,338**

[22] Filed: **Jun. 8, 1982**

Related U.S. Application Data

[63] Continuation of Ser. No. 75,192, Sep. 12, 1979, abandoned.

[51] **Int. Cl.**[3] **C12N 1/20; C12N 15/00; C12N 1/00; C12P 21/00**

[52] **U.S. Cl.** **435/253;** 435/68; 435/172; 435/317

[58] **Field of Search** 435/172, 253, 317

[56] **References Cited**

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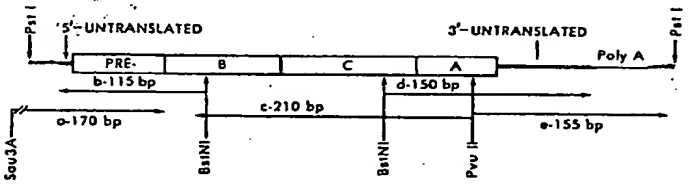
Primary Examiner—Alvin E. Tanenholtz
Attorney, Agent, or Firm—Keil & Witherspoon

[57] **ABSTRACT**

A DNA having a base sequence coding for human proinsulin and a DNA having a base sequence coding for human pre-proinsulin have been cloned, and novel recombinant DNA transfer vectors containing said cloned DNAs have been constructed. Novel microorganisms transformed by said recombinant transfer vectors have been obtained. Certain of said transformed microorganisms have demonstrated capability to express the cloned DNA's, synthesizing a protein comprising human proinsulin and a protein-comprising human pre-proinsulin.

14 Claims, 2 Drawing Figures



APPENDIX B

United States Patent [19]

Rutter et al.

[11] Patent Number: 4,652,525

[45] Date of Patent: Mar. 24, 1987

[54] RECOMBINANT BACTERIAL PLASMIDS CONTAINING THE CODING SEQUENCES OF INSULIN GENES

[75] Inventors: William J. Rutter; Raymond Pictet; John Chirgwin; Howard M. Goodman; Axel Ullrich; John Shine, all of San Francisco, Calif.

[73] Assignee: The Regents of the University of California, Berkeley, Calif.

[21] Appl. No.: 508,651

[22] Filed: Jun. 28, 1983

Related U.S. Application Data

[63] Continuation of Ser. No. 897,709, Apr. 19, 1978, abandoned, which is a continuation-in-part of Ser. No. 801,343, May 27, 1977, abandoned, and a continuation-in-part of Ser. No. 805,023, Jun. 9, 1977, abandoned.

[51] Int. Cl.[4] C12N 1/20; C12N 1/00; C12N 15/00; C12R 1/19

[52] U.S. Cl. 435/253; 435/317; 435/172.3; 435/849

[58] Field of Search 435/172.3, 68, 70, 71, 435/253, 317

[56] References Cited

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Primary Examiner—Alvin E. Tanenholtz

Attorney, Agent, or Firm—Ciotti & Murashige

[57] ABSTRACT

A recombinant procaryotic microorganism containing the gene coding for insulin.

7 Claims, No Drawings

Alternatively, the fusion protein is treated with a combination of trypsin and carboxypeptidase B (or cathepsin B) to yield active insulin from the fusion protein in a single reaction.

C. A proinsulin coding sequence is constructed by selective cleavage at an internal site in the proinsulin coding region, followed by ligation of a chemically synthesized sequence coding for that part of the proinsulin coding region removed by the previous cleavage. The plasmid pcHI-1 is used as a source of the proinsulin coding region, which is selectively excised by treatment with Pst I endonuclease or preferably by treatment with Hha I endonuclease, as described in Example 2B.

Either fragment, after isolation is treated with alkaline phosphatase to remove the 5' terminal phosphate groups, then cleaved by treatment with a restriction endonuclease having a unique cleavage point in the proinsulin coding sequence. Preferably the restriction site is located near one of the ends of the proinsulin coding sequence. The Alu I site in region of amino acids 13-14 provides a convenient cleavage point (see FIG. 2). The Hha I fragment of pcHI-1 is partially cleaved with Alu I endonuclease to generate two fragments of approximately 76bp and approximately 375bp, respectively. The Alu I fragments are fractionated by gel electrophoresis, as described in Example 2A, and the 375bp fragment is recovered.

A nucleotide sequence coding for the first 13 amino acids of proinsulin with a 5'-terminal G (on the plus strand), to complete the codon for alanine at position 14, is synthesized by the phosphotriester method, Itakura, K., et al. J. Biol. Chem. 250, 4592 (1975) and Itakura, K., et al., J. AM. Chem. Soc. 97, 7327 (1975). The plus strand of the synthetic DNA has the sequence 5'-TTTGTGAACCAACACCTG TGCGGCTCACACCTGGTGGAAG-3', corresponding to the natural sequence. However, other sequences coding for the same amino acids may be synthesized. In general the sequence is 5'-TTKGTLAAK-CAJCAKXTYTGKGGLQRSCAKXTYGTLCAJG-3'. The resulting sequence is blunt-end ligated with the approximately 375bp fragment of the Hba I fragment of pcHI-1. Since the latter has a 5'-phosphate only at the end to be joined, the two fragments will be joined in the correct order. The synethetic fragment is correctly joined to the larger fragment in approximately 50% of the reactions.

The ligase-treated DNA is then cloned into a suitable expression plasmid, either by oligo-A tailing, as described in Example 2B, or by attachment or linkers and insertion into expression plasmids of known reading frames. In the case of oligo-A trailed inserts, expression of proinsulin is observed in about 1/12 of the clones. In the case of direct insertion where the reading frame is known to be correct, the frequency of expression clones is about 50%.

EXAMPLE 3

Expression of human preproinsulin and proinsulin. The cloned inserts coding for preproinsulin (Example 1) or proinsulin (Example 2) are inserted in an expression transfer vector. When the insertion occurs in the correct orientation with respect to initiation of translation at the insertion site, and the insert is in reading frame phase with the promotor and ribosome binding site, the protein product of the cloned gene is synthesized by actively metabolizing host cells transformed by the transfer vector. The protein product is a fusion protein if the expression transfer vector contains a portion of a procaryotic gene between the promoter and the insertion site. However the insertion may be made immediately adjacent to a promoter site. In such cases, the protein coded by the insert is synthesized directly. Both techniques present advantages and disadvantages. Fusion proteins have the advantage that they tend to stabilize the foreign protein coded by the inserted gene. Also, desirable functional properties such as excretion from the host cell are conferred by fusion with certain host proteins such as β-lactamase. On the other hand, purification of the insert coded sequence is complicated by the general desirability of specifically removing the host portion of the fusion protein. Such removal is accomplished by known techniques as described in Examples 2A and 2B. Direct synthesis of the desired protein obviates the need for specific cleavage but generally precludes the possibility of excretion from the host cell.

Expression plasmids have been developed wherein expression is controlled by the lac promoter (Itakura, et al., Science 198, 1056 (1977), Ullrich, A., et al., Excerpta Medica, (1979); by the trp promoter (Martial, et al., Science 205, 602 (1979); and by the β-lactamase promoter, U.S. application Ser. No. 44,647, incorporated herein by reference.

Expression is detected by measurement of a product capable of binding immunochemically with anti-insulin antibody, or anti-proinsulin antibody. Radioimmunoassay, in which the antibody is radioactively labeled and antigen-antibody pairs are precipitated by a preparation of heat-killed Staphyloccoccus aureus C is employed. (See Morgan and Lazarow, Diabetes 12, 115 (1963) and Kessler, S. W., J. Immunol., 115, 1617 (1975). Radioimmune screening, as described by Erlich, H. A., et al., Cell 10, 681 (1978) or by Broome, S. and Gilbert, W., Proc. Nat. Acad. Sci. USA, 75, 2746 (1978), is used for detecting expression in bacterial colonies.

Fusion proteins indicative of expression are detected by comparing molecular weights of the host protein contributing the N-terminal part of the fusion protein, in host cells transformed by expression plasmids with and without an insert. A preferred variant is to employ the minicell-producing *E. coli* strain P678-54 as host. Radioactively labeled amino acids are incorporated into minicell proteins, comparing strains transformed with expression transfer vectors with and without the inserted proinsulin coding sequence. The proteins are fractionated by SDS-acrylamide gel electrophoresis and the protein positions detected by autoradiography. Expression of proinsulin is indicated by the presence of a labeled protein band found only in minicells transformed by the proinsulin expression plasmid. The position of the electrophoretic band provides a measure of the molecular weight of the expressed protein, and is consistent with the known length of the inserted gene and of the N-terminal procaryotic portion.

Removal of the procaryotic portion and conversion of proinsulin to insulin in vitro are carried out by known procedures, as described in detail supra.

What is claimed is:

1. A DNA transfer vector comprising an inserted cDNA consisting essentially of a deoxynucleotide sequence coding for human pre-proinsulin, the plus strand of said cDNA having a defined 5' end, said 5' end being the first deoxynucleotide of the sequence coding for said pre-proinsulin.

2. A DNA transfer vector comprising an inserted cDNA consisting essentially of a deoxynucleotide se-

quence coding for human proinsulin, the plus strand of said cDNA having a defined 5' end, said 5' end being the first deoxynucleotide of the sequence coding for said proinsulin.

3. A microorganisms transformed by the transfer vector of claim 1 or 2.

4. A DNA transfer vector comprising a deoxynucleotide sequence coding for human pre-proinsulin consisting essentially of a plus strand having the sequence:

5'-$_{-24}$GCL$_{-23}$X$_{-22}$TY$_{-22}$TGG$_{-21}$ATG$_{-20}$W$_{-19}$GZ$_{-19}$X$_{-18}$TY$_{-18}$X$_{-17}$TY$_{-17}$CCL$_{-16}$X$_{-15}$TY$_{-15}$X$_{-14}$TY$_{-14}$GCL$_{-13}$X$_{-12}$TY$_{-12}$X$_{-11}$TY$_{-11}$GCL$_{-10}$X$_{-9}$TY$_{-9}$TGG$_{-8}$GGL$_{-7}$CCL$_{-6}$GAK$_{-5}$CCL$_{-4}$GCL$_{-3}$GCL$_{-2}$GCL$_{-1}$TTK$_{1}$GTL$_{2}$AAK$_{3}$CAJ$_{4}$CAK$_{5}$X$_{6}$TY$_{6}$TGK$_{7}$GGL$_{8}$QR$_{9}$S$_{9}$CAK$_{10}$X$_{11}$TY$_{11}$GTL$_{12}$GAJ$_{13}$GCL$_{14}$X$_{15}$TY$_{15}$TAK$_{16}$X$_{17}$TY$_{17}$GTL$_{18}$TGK$_{19}$GCL$_{20}$GAJ$_{21}$W$_{22}$GZ$_{22}$GCL$_{23}$TTK$_{24}$TTK$_{25}$TAK$_{26}$ACL$_{27}$CCL$_{28}$AAJ$_{29}$ACL$_{30}$W$_{31}$GZ$_{31}$W$_{32}$GZ$_{32}$GAJ$_{33}$GCL$_{34}$GAJ$_{35}$GAK$_{36}$X$_{37}$TY$_{37}$CAJ$_{38}$GTL$_{39}$GGL$_{40}$CAJ$_{41}$GTL$_{42}$GAJ$_{43}$X$_{44}$TY$_{44}$GGL$_{45}$GGL$_{46}$GGL$_{47}$CCL$_{48}$GGL$_{49}$GCL$_{50}$GGL$_{51}$QR$_{52}$S$_{52}$X$_{53}$TY$_{53}$CAJ$_{54}$CCL$_{55}$X$_{56}$TY$_{56}$GCL$_{57}$X$_{58}$TY$_{58}$GAJ$_{59}$GGL$_{60}$QR$_{61}$S$_{61}$X$_{62}$TY$_{62}$CAJ$_{63}$AAJ$_{64}$W$_{65}$GZ$_{65}$GGL$_{66}$ATM$_{67}$GTL$_{68}$GAJ$_{69}$CAJ$_{70}$TGK$_{71}$TGK$_{72}$ACL$_{73}$QR$_{74}$S$_{74}$ATM$_{75}$TGK$_{76}$QR$_{77}$S$_{77}$X$_{78}$TY$_{78}$TAK$_{79}$CAJ$_{80}$X$_{81}$TY$_{81}$GAJ$_{82}$AAK$_{83}$TAK$_{84}$TGK$_{85}$AAK$_{86}$TAGACGCAGCCCGCAGGCAGCCCCCCACCCGCCGCCTCCTGCACCGAGAGAGATGGAATAAAGCCCTTGAACCAGC poly A-3' wherein

A is deoxyadenyl,
G is deoxyguanyl,
C is deoxycytosyl,
T is thymidyl,
J is A or G;
K is T or C;
L is A, T, C, or G;
M is A, C or T;
X_n is T or C if Y_n is A or G; and C if Y_n is C or T;
Y_n is A, G, C or T if X_n is C, and A or G if X_n is T;
W_n is C or A if Z_n is G or A, and C if Z_n is C or T;
Z_n is A, G, C or T if W_n is C, and A or G if W_n is A;
QR_n is TC if S_n is A, G, C or T, and AG if S_n is T or C;
S_n is A, G, C or T if QR_n is TC, and T or C if QR_n is AG; and, subscript numerals, n, refer to the position in the amino acid sequence of human proinsulin, to which each triplet in the nucleotide sequence corresponds, according to the genetic code, the amino acid positions being numbered from the amino end.

5. A DNA transfer vector comprising a deoxynucleotide sequence coding for human proinsulin consisting essentially of a plus strand having the sequence:

5'-TTK$_{1}$GTL$_{2}$AAK$_{3}$CAJ$_{4}$CAK$_{5}$X$_{6}$TY$_{6}$TGK$_{7}$GGL$_{8}$QR$_{9}$S$_{9}$CAK$_{10}$X$_{11}$TY$_{11}$GTL$_{12}$GAJ$_{13}$GCL$_{14}$X$_{15}$TY$_{15}$TAK$_{16}$X$_{17}$TY$_{17}$GTL$_{18}$TGK$_{19}$GCL$_{20}$GAJ$_{21}$W$_{22}$GZ$_{22}$GCL$_{23}$TTK$_{24}$TTK$_{25}$TAK$_{26}$ACL$_{27}$CCL$_{28}$AAJ$_{29}$ACL$_{30}$W$_{31}$GZ$_{31}$W$_{32}$GZ$_{32}$GAJ$_{33}$GCL$_{34}$GAJ$_{35}$GAK$_{36}$X$_{37}$TY$_{37}$CAJ$_{38}$GTL$_{39}$GGL$_{40}$CAJ$_{41}$GTL$_{42}$GAJ$_{43}$X$_{44}$TY$_{44}$GGL$_{45}$GGL$_{46}$GGL$_{47}$CCL$_{48}$GGL$_{49}$GCL$_{50}$GGL$_{51}$QR$_{52}$S$_{52}$X$_{53}$TY$_{53}$CAJ$_{54}$CCL$_{55}$X$_{56}$TY$_{56}$GCL$_{57}$X$_{58}$TY$_{58}$GAJ$_{59}$GGL$_{60}$QR$_{61}$S$_{61}$X$_{62}$TY$_{62}$CAJ$_{63}$AAJ$_{64}$W$_{65}$GZ$_{65}$GGL$_{66}$ATM$_{67}$GTL$_{68}$GAJ$_{69}$CAJ$_{70}$TGK$_{71}$TGK$_{72}$ACL$_{73}$QR$_{74}$S$_{74}$ATM$_{75}$TGK$_{76}$QR$_{77}$S$_{77}$X$_{78}$TY$_{78}$TAK$_{79}$CAJ$_{80}$X$_{81}$TY$_{81}$GAJ$_{82}$AAK$_{83}$TAK$_{84}$TGK$_{85}$AAK$_{86}$TAG-3' wherein

A is deoxyadenyl,
G is deoxyguanyl,
C is deoxycytosyl,
T is thymidyl,
J is A or G;
K is T or C;
L is A, T, C, or G;
M is A, C or T;
X_n is T or C if Y_n is A or G; and C if Y_n is C or T;
Y_n is A, G, C or T if X_n is C, and A or G if X_n is T;
W_n is C or A if Z_n is G or A, and C if Z_n is C or T;
Z_n is A, G, C or T if W_n is C, and A or G if W_n is A;
QR_n is TC if S_n is A, G, C or T, and AG if S_n is T or C;
S_n is A, G, C or T if QR_n is TC, and T or C if QR_n is AG; and, subscript numerals, n, refer to the position in the amino acid sequence of human proinsulin, to which each triplet in the nucleotide sequence corresponds, according to the genetic code, the amino acid positions being numbered from the amino end.

6. A microorganism transformed by the transfer vector of claim 4 or 5.

7. The plasmid pcHI-1.

8. The plasmid pcHP-1.

9. A microorganism transformed by the plasmid of claim 7 or 8.

10. A microorganism as in claim 9 wherein the organism is *Escherichia coli.*

11. The microorganism as in claim 10 wherein the organism is *Escherichia coli* HB-101.

12. The DNA transfer vector of claim 4 wherein:
J is A in amino acid positions 4, 13, 21, 69 and 70;
J is G in amino acid positions 29, 33, 34, 38, 41, 43, 54, 59, 63, 64, 80 and 82;
K is T in amino acid positions 1 and 72;
K is C in amino acid positions −5, 3, 5, 7, 10, 16, 19, 24, 25, 26, 36, 71, 76, 79, 83, 84, 85 and 86;
L is A in amino acid positions −7, −4, −2, 27, 34 and 50;
L is T in amino acid positions −6, 14, 48 and 49;
L is C in amino acid positions −23, −16, −10, −3, −1, 8, 23, 28, 30, 45, 47, 51, 55, 57, 66 and 73;
L is G in amino acid positions −13, 2, 12, 18, 20, 39, 40, 42, 46, 60 and 68;
M is C in amino acid position 75;
M is T in amino acid position 67;
X is T in amino acid position 56;
X is C in amino acid positions −22, −18, −17, −15, −14, −12, −11, −9, 6, 11, 15, 17, 37, 53, 58, 62, 78 and 81;
X is G in amino acid position 44;
Y is A in amino acid position 17;
Y is G in amino acid positions −22, −17, −15, −14, −12, −11, −9, 6, 11, 37, 44, 53, 56, 58, 62 and 81;
Y is C in amino acid positions −18, 15 and 78;
W is C in amino acid positions −19, 22, 31, 32 and 65;
Z is C in amino acid position −19;
Z is A in amino acid position 22;
Z is G in amino acid positions 31 and 32;
Z is T in amino acid position 65;
QR is TC in amino acid positions 9, 62 and 77;
QR is AG in amino acid positions 52 and 74;
S is A in amino acid position 9; and
S is C in amino acid positions 52, 61, 74 and 77.

13. The DNA transfer vector of claim 5 wherein:

J is A in amino acid positions 4, 13, 21, 69 and 70;
J is G in amino acid positions 29, 33, 35, 38, 41, 43, 54, 59, 63, 64, 80 and 82;
K is T in amino acid positions 1 and 72;
K is C in amino acid positions 3, 5, 7, 10, 16, 19, 24, 25, 26, 36, 71, 76, 79, 83, 84, 85 and 86;
L is A in amino acid positions 27, 34 and 50;
L is T in amino acid positions 14, 48 and 49;
L is C in amino acid positions 8, 23, 28, 30, 45, 47, 51, 55, 57, 66 and 73;
L is G in amino acid positions 2, 12, 18, 20, 39, 40, 42, 46, 60 and 68;
M is C in amino acid position 75;
M is T in amino acid position 67;
X is T in amino acid position 56;
X is C in amino acid positions 6, 11, 15, 17, 37, 53, 58, 62, 78 and 81;
X is G in amino acid position 44;
Y is A in amino acid position 17;
Y is G in amino acid positions 6, 11, 37, 44, 53, 56, 58, 62 and 81;
Y is C in amino acid positions 15 and 78;
W is C in amino acid positions 22, 31, 32 and 65;
Z is A in amino acid position 22;
Z is G in amino acid positions 31 and 32;
Z is T in amino acid position 65;
QR is TC in amino acid positions 9, 62 and 77;
QR is AG in amino acid positions 52 and 74;
S is A in amino acid position 9; and
S is C in amino acid positions 52, 61, 74 and 77.

14. The DNA transfer vector of claim 5 wherein the codon for amino acid position one is preceded by 5'-ATG and

J is A in amino acid positions 13, 21, 69 and 70;
J is G in amino acid positions 4, 29, 33, 35, 38, 41, 43, 54, 59, 63, 64, 80 and 82;
K is T in amino acid positions 3, 7 and 72;
K is C in amino acid positions 1, 5, 10, 16, 19, 24, 25, 26, 36, 71, 76, 79, 83, 84, 85 and 86;
L is A in amino acid positions 27, 34, 50;
L is T in amino acid positions 8, 12, 14, 48 and 49;
L is C in amino acid positions 2, 23, 28;
30, 45, 47, 51, 55, 57, 66 and 73;
L is G in amino acid positions 18, 20, 39, 40, 42, 46, 60 and 68;
M is C in amino acid position 75;
M is T in amino acid position 67;
X is T in amino acid position 56;
X is C in amino acid positions 6, 11, 15, 17, 37, 53, 58, 62, 78, and 81;
X is G in amino acid position 44;
Y is A in amino acid position 17;
Y is G in amino acid positions 37, 44, 53, 56, 58, 62, and 81;
Y is C in amino acid positions 11, 15 and 78;
Y is T in amino acid position 6;
W is C in amino acid positions 22, 31, 32 and 65;
Z is A in amino acid position 22;
Z is G in amino acid positions 31 and 32;
Z is T in amino acid position 65;
QR is TC in amino acid positions 9, 62 and 77;
QR is AG in amino acid positions 52 and 74
S is T in amino acid position 9; and
S is C in amino acid positions 52, 61, 74 and 77.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,431,740

DATED : FEBRUARY 14, 1984

INVENTOR(S) : GRAEME BELL ET AL

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Column 17:

Claim 4, line 11 of GCL_{20}" should read --GGL_{20}--;

Claim 4, line 12, "CL_{23}" should read --GL_{23}--;

Claim 5, line 6, "GCL_{20}" should read GGL_{20}--;

Claim 5, line 7, "GCL_{23}" should read --GGL_{23}--;

Column 15, Claim 12, line 3, "34" should read —35—.
Claim 12, line 31 "QR is TC in amino acid positions 9, 62 and 77; should read —QR is TC in amino acid positions 9, 61 and 77;—

Column 19, Claim 13, line 28 "QR" is TC in amino acid positions 9, 61 and 77;—

Column 20, Claim 14, line 31 "QR is TC in amino acid positions 9, 62 and 77;" should read —QR is TC is amino acid positions 9, 61 and 77;—.

Signed and Sealed this

Twenty-fifth Day of January, 1994

Attest:

Bruce Lehman

Attesting Officer

BRUCE LEHMAN

Commissioner of Patents and Trademarks

clones was carried out on plates containing 20 μg/ml ampicillin.

EXAMPLE 5

The DNA from pAU-1 as described in Example 4 was further purified by electrophoresis on a 6% polyacrylamide gel. After elution from the gel the DNA was labeled by incubation with γ-^{32}P-ATP and the enzyme polynucleotide kinase under conditions described by Maxam and Gilbert, supra. The enzyme catalyzes the transfer of a radiactive phosphate group from γ-^{32}P-ATP to the 5'-ends of the DNA. The enzyme was obtained from *E. coli* by the method of Panet, A., et al., *Biochemistry* 12, 5045 (1973). The DNA thus labeled was cleaved with Hae III endonuclease as described in Example 2, and the two labeled fragments, about 265 and 135 base pairs respectively, were separated on a polyacrylamide gel under the conditions described in Example 1. The isolated fragments were subjected to specific cleavage reactions and sequence analysis according to the method of Maxam and Gilbert, supra. The sequence below is based upon a composite of the findings from this series of experiments and those of a similar series of cDNA using plasmid vectors derived from col E1 such as pMB9 and pBR322. In the sequence of the 5' end, a sequence estimated between 50–120 nucleotides in length is undetermined and the poly dA segment at the 3'-end is of varying length. This sequence is provided as representing the best information presently available, with the understanding that ongoing studies may reveal additional details or may indicate a need for slight revision in some areas. The corresponding amino acid sequence of rat proinsulin I begins at the triplet position marked 1 and ends at triplet position marked 86. Some uncertainty remains with respect to the sequence underlined with a dashed line.

[undetermined] —GCC CTG CTC GTC CTC TGG GAG CCC AAG CCT GCT CAG GCT TTT GTC AAA CAG CAC CTT TGT
GGT CCT CAC CTG GTG GAG GCT CTG TAC CTG GTG TGT GGG GAA CGT GGT TTC TTC TAC ACA CCC AAC
TCC CGT CGT GAA GTG GAG GAC CCG CAA GTG CCA CAA CTG GAG CTG GCT GGA GGC CCG GAG GCC GGG
GAT CTT CAG ACC TGG GCA CTG GAG GTT GCC CGG CAG AAG CGT GGC ATT GTG GAT CAG TGC TGC ACC
AGC ATC TGC TCC CTC TAC CAA CTG CAG AAC TAC TGC AAC TGA
GTTCAATCAATTCCCGATCCACCCCTCTGCAATGAATAAAGCCTTTGAATGAGC-poly A
Scored Sections = Areas of Present Uncertainty

EXAMPLE 6

A nucleotide sequence coding for human insulin is isolated, purified and incorporated in a plasmid essentially as described in Examples 1–4, starting from human pancreas tissue isolated from a suitable human source such as a donated pancreas or a fresh cadaver or a human insulinoma. A microorganism is produced, essentially as described in Example 4, having a nucleotide sequence coding for the human insulin A chain and B chain. The known amino acid sequence of human insulin A chain is:

Gly—Ile—Val—Glu—Gln—Cys—Cys—Thr—Ser—Ile—
—Cys—Ser—Leu—Tyr—Glu—Leu—Glu—Asn—
—Tyr—Cys—Asn

The known amino acid sequence of the human insulin B chain is:

Phe—Val—Asn—Glu—His—Leu—Cys—Gly—Ser—His—
—Leu—Val—Glu—Ala—Leu—Tyr—Leu—Val—Cys—Gly—
—Glu—Arg—Gly—Phe—Phe—Tyr—Thr—Pro—Lys—Thr

The amino acid sequences are numbered from the end having a free amino group. See Smith, L. F., *Diabetes* 21 (suppl. 2), 458 (1972).

GENERAL CONCLUDING REMARKS

With the process of the present invention it has become possible for the first time to isolate a nucleotide sequence coding for a specific regulatory protein from a higher organism such as a vertebrate, and transfer the genetic information contained therein to a microorganism where it may be replicated indefinitely. The disclosed process may be applied to the isolation and purification of the human insulin gene, and to its transfer to a microorganism. A novel recombinant plasmid is disclosed, containing within its nucleotide sequence a subsequence having the structure of and transcribed from a gene of a higher organism. A novel microorganism is disclosed, modified to contain a nucleotide sequence having the structure of and transcribed from a gene of a higher organism. The practice of the invention has been illustrated by demonstrating the transfer of the rat gene for the proinsulin I to a strain of *Escherichia coli*. The sequence of the main portion of the transferred gene has been determined and has been found to contain the entire amino acid sequence of rat proinsulin I, as determined by reference to the known genetic code which is common to all forms of life.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains or which would be readily apparent to those skilled in said art. With that understanding, the invention is not to be limited except to the extent required by the appended claims.

What is claimed is:

1. A recombinant plasmid replicable in procaryotic host containing within its nucleotide sequence a subsequence having the structure of the reverse transcript of an mRNA of a vertebrate, which mRNA encodes insulin.

56. An isolated DNA molecule in accordance with claim 52, comprising the nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

i. includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and

ii. the ability to inhibit the cytotoxic effect of TNF-α on murine A9 cells.

57. A replicable expression vehicle comprising the DNA molecule of claim 56 and capable, in a transformant host cell, of expressing said protein.

58. A host cell selected from the group consisting of a prokaryotic and a eukaryotic cell transformed with the replicable expression vehicle of claim 57.

59. An isolated DNA molecule comprising the nucleotide sequence coding for a naturally occurring human Tumor Necrosis Factor (TNF) binding protein (TBP-II) having the following characteristics:

i. includes the amino acid sequence Thr-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-Ser-Thr in the portion of the protein sequenced by N-terminal sequence analysis; and